REMARKS

Applicants request that the Amendment After Final Rejection filed February 15, 2006, not be entered, and that the present amendments constitute the necessary Submission for the concurrently filed RCE Transmittal.

Applicants have amended their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants have amended claim 8 to recite a method for gene expression analysis of genes derived from different samples; to recite that the first and second samples utilized respectively in preparing the first and second nucleotides are different from each other; to recite the step of mixing the first and second nucleotides, prepared as recited in the present claims; and to correct a typographical error. These are amendments as set forth in the Amendment After Final Rejection filed February 15, 2006.

Moreover, Applicants are adding new claim 12 to the application. Claim 12, dependent on claim 8, recites that the samples are from different specimens.

Note, for example, the first full paragraph on page 5 of Applicants' specification. Note also, for example, Example 2 described on pages 19 and 20 of Applicants' specification.

Applicants have cancelled non-elected claims 6 and 7 without prejudice or disclaimer, and in particular without prejudice to the filing of a Divisional application directed to the subject matter thereof.

Noting concurrent filing of the RCE Transmittal, clearly entry of the present amendments is proper.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner, patentably distinguish over the teachings of the references applied by the Examiner in rejecting claims in the Office Action mailed

November 15, 2005, that is, the teachings of the U.S. Patent to Shah, et al., No. 6,165,723, and International (PCT) Publication No. WO 97/42345 (Whitcombe, et al.), under the provisions of 35 USC 102 and 35 USC 103.

It is respectfully submitted that the references as applied by the Examiner, either alone or in combination, would have neither taught nor would have suggested such a method for gene expression analysis as in the present claims, including, inter alia, preparing first nucleotides including a targeted gene by using a first sample and preparing second nucleotides including this targeted gene by using a second sample, the first and second samples being different from each other, and introducing various base sequences which are nonspecific to the base sequence of the targeted gene, to the targeted gene so that the various base sequences are bound at recited positions relative to the 5' end; mixing the first and second nucleotides; and subjecting the first and second nucleotides to nucleic acid amplification using various primers and probes, and thermostable DNA polymerase having specified activity, with digesting of the first and second probes and detecting a fluorescence emitted by respective fluorophores released in digesting the first and second probes, to thereby assay the amount of the product of the nucleic acid amplification. Note claim 8.

It is emphasized that according to the present invention, a gene expression analysis method for at least two genes provided from different samples, e.g., from different specimens (see claim 12) can be performed. As will be shown infra, it is respectfully submitted that the primary reference applied by the Examiner, Whitcombe, et al., would have neither disclosed nor would have suggested such gene expression analysis method as in the present claims, of genes derived from

different samples, Whitcombe, et al. describing at most the same samples being analyzed.

In addition, it is respectfully submitted that the teachings of the applied references would have neither disclosed nor would have suggested such method for gene expression analysis as in the present claims, having features as discussed previously in connection with claim 8, and, additionally, the further definition of synthesizing the first and second nucleotides as recited in claim 9; and/or the further definition of the first and second nucleotides as in claim 10; and/or wherein the Tm values of the first and second probes are substantially the same, as in claim 11; and/or wherein the samples come from different specimens (see claim 12).

According to the present invention, first and second nucleotides are prepared respectively from first and second samples which are different from each other, and, e.g., are from different specimens, and respectively are subjected to nucleic acid amplification using first and second probes respectively having a base sequence identical or complementary to base sequences used in preparing the first and second nucleotides, respectively, with each of the first and second probes being labeled at one end with a respective fluorophore and at another end with a quencher. Such technique as in the present invention enables highly accurate quantitative analysis of expression levels of a targeted gene in two or more samples, which are different from each other, under substantially the same conditions by real-time polymerase chain reaction (PCR). Note, for example, the sole full paragraph on page 12, the second paragraph on page 15, and the sole paragraph on page 22, of Applicants' specification.

Thus, to be illustrative and not to be limiting, the method of the present invention is used for, e.g., simultaneous analysis of genes derived from several

different samples, e.g., from different specimens (for example, GAPDH derived from a liver and GAPDH derived from a kidney as described in Example 2 starting from page 19 of Applicants' specification).

Whitcombe, et al. discloses a method for the detection of diagnostic base sequences in sample nucleic acid, using tailed diagnostic primers having a tag region and a detector region. The method includes contacting a sample under hybridizing conditions and in the presence of appropriate nucleoside triphosphates and an agent for polymerization thereof, with a diagnostic primer for the diagnostic base sequence, the diagnostic base primer having a tail sequence comprising a tag region and a detector region, such that an extension product of the diagnostic primer is synthesized when the corresponding diagnostic base sequence is present in the sample, no extension product being synthesized when the corresponding diagnostic base sequence is not present in the sample and any extension product of the diagnostic primer acting as a template for extension of a further primer which hybridizes to a locus at a distance from the diagnostic base sequence; contacting the sample with a tag primer which selectively hybridizes to the complement of the tag sequence in an extension product of the further primer and is extended; and detecting the presence or absence of the diagnostic base sequence by reference to the detector region in the further primer extension product. Note the paragraph bridging pages 1 and 2 of this patent. Note also page 4, lines 22-27, disclosing use of diagnostic and further primers which are genome specific at their 3'-termini but which carry a detector region and common extensions (tags) at their 5'-termini. Note also from page 6, line 24 through page 7, line 1; and page 10, lines 7-9, of Whitcombe, et al.

As can be seen in the foregoing, as well as from a full review of Whitcombe, et al., it is respectfully submitted that this reference does not disclose, nor would have suggested, such method as in the present claims, including the preparation of first and second nucleotides including the targeted gene from first and second samples, which first and second samples are different from each other, together with the other processing as in the present claims, and advantages thereof as discussed in the foregoing; and/or other features of the present invention as discussed previously, and advantages thereof.

It is respectfully submitted that Whitcombe, et al. describes an expression analysis of two or more genes derived from one sample (that is, the same sample). It is respectfully submitted that the method described in Whitcombe, et al. is intended to be used for identifying a very small fraction of a variant sequence in a normal sequence (for example, cancer diagnosis as described on page 6, lines 24-29 of Whitcombe, et al.), or detecting the presence or absence of more than one suspected variant nucleotide in the same sample (see, for example, page 7, lines 6 and 7, of Whitcombe, et al.). For example, in the diagnosis of cancer, the expression analysis of Whitcombe, et al. is performed by a two-stage amplification procedure comprising a first stage to amplify any variant sequence that may be present using primers of the Amplification Refractory Mutation System, and a second stage to perform a genomic controlling reaction in the same reaction vessel using the same primers at low concentrations (note, for example, page 6, lines 24-29 of Whitcombe, et al.). Thus, variant sequences and a normal sequence derived from the same sample are identified.

Note also the following description at page 7, lines 6 and 7 of Whitcombe, et al.:

> "A further and important use of [the Amplification Refractory Mutation System] is for detecting the presence or absence of more than one suspected variant nucleotide in the same sample. [Emphasis added.]"

Thus, it is respectfully submitted that Whitcombe, et al. is clearly concerned with analysis of one sample.

The Examiner has referred to Fig. 17 of Whitcombe, et al., in connection the claimed subject matter, the Examiner asserting that Whitcombe, et al. discloses preparing first nucleotides including a targeted gene by using a first sample (allele A) and preparing second nucleotides including the targeted gene by using a second sample (allele B). It is respectfully submitted, however, that this is an incorrect interpretation of the teachings of Whitcombe, et al., and, in particular, an incorrect interpretation of Fig. 17. It is respectfully submitted that Fig. 17 of Whitcombe, et al. shows single-tube genotyping using primers of the Amplification Refractory Mutation System for each target sequence, that is, allele A and allele B. It is respectfully submitted that allele A and allele B both exist is a single sample derived from the same subject; and it is respectfully submitted that, therefore, Fig. 17 only shows genotyping for each allele derived from one sample.

As set forth previously, it is respectfully submitted that the method according to the present invention differs from the method described in Whitcombe, et al., in that the method of the present invention is a method for gene expression analysis of genes derived from different samples, e.g., from different specimens, whereas the method described in Whitcombe, et al. is an expression analysis of genes derived

from one sample. Due to this difference, the methods can be used for different purposes, as discussed in the foregoing.

Even assuming, arguendo, that the teachings of Shah, et al. were properly combinable with the teachings of Whitcombe, et al., it is respectfully submitted that such combined teachings would have neither disclosed nor would have suggested the presently claimed invention.

Shah, et al. discloses a method for detecting a target nucleic acid fragment directly from a specimen obtained from a patient by in situ hybridization, the method including steps in the listed order of:

(1) Depositing a sample of the specimen onto a slide;

(2) Fixing the sample onto the slide with fixative, the fixative comprising either methanol-acetic acid at a ratio of from 99:1 to 80:20, or formalin-acetic acid at a ratio of from 99:1 to 80:20;

(3) Contacting the nucleic acids of affixed sample with a probe complex specific for the target nucleic acid fragment, under conditions appropriate for hybridization;

(4) Rinsing non-hybridized probe complex from the sample and staining the rinsed sample with Evans Blue; and

(5) Visually detecting the hybridized probe complex by microscopy, with the presence of the probe complex being an indication of the presence of the target nucleic acid fragment.

See column 1, lines 38-54. See also column 2, lines 4-8, 29-33 and 53-56. Note further, column 4, lines 58-63; and column 5, lines 43-47.

Even assuming, arguendo, that the teachings of Shah, et al. were properly combinable with the teachings of Whitcombe, et al., such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, the preparation of the first and second nucleotides each including the targeted gene by using first and second samples which are different

from each other, respectively, as in claim 8; and/or the other features of the present invention as discussed previously, and advantages thereof.

Reference by the Examiner to Overbergh, et al., in the paragraph bridging pages 4 and 5 of the Office Action mailed November 15, 2005, is noted. Reliance thereon is improper without including this reference in the statement of the rejection. See In re Hoch, 166 USPQ 406, 407 n.3 (CCPA 1970). It is respectfully submitted that a new rejection in a new Office Action is required, if Overbergh, et al. is being relied upon by the Examiner.

In view of the foregoing comments and amendments, entry of the present amendments, in light of the concurrently filed RCE Transmittal, and reconsideration and allowance of all claims presently pending in the above-identified application, are respectfully requested.

Applicants request any shortage of fees due in connection with the filing of this paper be charged to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Deposit Account No. 01-2135 (case 1021.43085X00), and credit any excess payment of fees to such Deposit Account.

Respectfully submitted,

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